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**Panzer et al.**(10) **Pub. No.: US 2004/0048253 A1**(43) **Pub. Date: Mar. 11, 2004**(54) **MOLECULES FOR DIAGNOSTICS AND THERAPEUTICS**

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(57) **ABSTRACT**

The present invention provides purified human polynucleotides for diagnostics and therapeutics (dithp). Also encompassed are the polypeptides (DITHP) encoded by dithp. The invention also provides for the use of dithp, or complements, oligonucleotides, or fragments thereof in diagnostic assays. The invention further provides for vectors and host cells containing dithp for the expression of DITHP. The invention additionally provides for the use of isolated and purified DITHP to induce antibodies and to screen libraries of compounds and the use of anti-DITHP antibodies in diagnostic assays. Also provided are microarrays containing dithp and methods of use.

of predisposition for a particular condition, disease, or disorder. For example, cardiovascular disease may result from malfunctioning receptor molecules that fail to clear cholesterol from the bloodstream, and diabetes may result when a particular individual's immune system is activated by an infection and attacks the insulin-producing cells of the pancreas. In some studies, Alzheimer's disease has been linked to a gene on chromosome 21; other studies predict a different gene and location. Mapping of disease genes is a complex and reiterative process and generally proceeds from genetic linkage analysis to physical mapping.

[0717] As a condition is noted among members of a family, a genetic linkage map traces parts of chromosomes that are inherited in the same pattern as the condition. Statistics link the inheritance of particular conditions to particular regions of chromosomes, as defined by RFLP or other markers. (See, for example, Lander, E. S. and Botstein, D. (1986) *Proc. Natl. Acad. Sci. USA* 83:7353-7357.) Occasionally, genetic markers and their locations are known from previous studies. More often, however, the markers are simply stretches of DNA that differ among individuals. Examples of genetic linkage maps can be found in various scientific journals or at the Online Mendelian Inheritance in Man (OMIM) World Wide Web site.

[0718] In another embodiment of the invention, dithp sequences may be used to generate hybridization probes useful in chromosomal mapping of naturally occurring genomic sequences. Either coding or noncoding sequences of dithp may be used, and in some instances, noncoding sequences may be preferable over coding sequences. For example, conservation of a dithp coding sequence among members of a multi-gene family may potentially cause undesired cross hybridization during chromosomal mapping. The sequences may be mapped to a particular chromosome, to a specific region of a chromosome, or to artificial chromosome constructions, e.g., human artificial chromosomes (HACs), yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial P1 constructions, or single chromosome cDNA libraries. (See, e.g., Harrington, J. J. et al. (1997) *Nat. Genet.* 15:345-355; Price, C. M. (1993) *Blood Rev.* 7:127-134; and Trask, B. J. (1991) *Trends Genet.* 7:149-154.)

[0719] Fluorescent in situ hybridization (FISH) may be correlated with other physical chromosome mapping techniques and genetic map data. (See, e.g., Meyers, supra, pp. 965-968.) Correlation between the location of dithp on a physical chromosomal map and a specific disorder, or a predisposition to a specific disorder, may help define the region of DNA associated with that disorder. The dithp sequences may also be used to detect polymorphisms that are genetically linked to the inheritance of a particular condition, disease, or disorder.

[0720] In situ hybridization of chromosomal preparations and genetic mapping techniques, such as linkage analysis using established chromosomal markers, may be used for extending existing genetic maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse, may reveal associated markers even if the number or arm of the corresponding human chromosome is not known. These new marker sequences can be mapped to human chromosomes and may provide valuable information to investigators searching for disease genes using positional

cloning or other gene discovery techniques. Once a disease or syndrome has been crudely correlated by genetic linkage with a particular genomic region, e.g., ataxia-telangiectasia to 11q22-23, any sequences mapping to that area may represent associated or regulatory genes for further investigation. (See, e.g., Gatti, R. A. et al. (1988) *Nature* 336:577-580.) The nucleotide sequences of the subject invention may also be used to detect differences in chromosomal architecture due to translocation, inversion, etc., among normal, carrier, or affected individuals.

[0721] Once a disease-associated gene is mapped to a chromosomal region, the gene must be cloned in order to identify mutations or other alterations (e.g., translocations or inversions) that may be correlated with disease. This process requires a physical map of the chromosomal region containing the disease-gene of interest along with associated markers. A physical map is necessary for determining the nucleotide sequence of and order of marker genes on a particular chromosomal region. Physical mapping techniques are well known in the art and require the generation of overlapping sets of cloned DNA fragments from a particular organelle, chromosome, or genome. These clones are analyzed to reconstruct and catalog their order. Once the position of a marker is determined, the DNA from that region is obtained by consulting the catalog and selecting clones from that region. The gene of interest is located through positional cloning techniques using hybridization or similar methods.

#### [0722] Diagnostic Uses

[0723] The dithp of the present invention may be used to design probes useful in diagnostic assays. Such assays, well known to those skilled in the art, may be used to detect or confirm conditions, disorders, or diseases associated with abnormal levels of dithp expression. Labeled probes developed from dithp sequences are added to a sample under hybridizing conditions of desired stringency. In some instances, dithp, or fragments or oligonucleotides derived from dithp, may be used as primers in amplification steps prior to hybridization. The amount of hybridization complex formed is quantified and compared with standards for that cell or tissue. If dithp expression varies significantly from the standard, the assay indicates the presence of the condition, disorder, or disease. Qualitative or quantitative diagnostic methods may include northern, dot blot, or other membrane or dip-stick based technologies or multiple-sample format technologies such as PCR, enzyme-linked immunosorbent assay (ELISA)-like, pin, or chip-based assays.

[0724] The probes described above may also be used to monitor the progress of conditions, disorders, or diseases associated with abnormal levels of dithp expression, or to evaluate the efficacy of a particular therapeutic treatment. The candidate probe may be identified from the dithp that are specific to a given human tissue and have not been observed in GenBank or other genome databases. Such a probe may be used in animal studies, preclinical tests, clinical trials, or in monitoring the treatment of an individual patient. In a typical process, standard expression is established by methods well known in the art for use as a basis of comparison, samples from patients affected by the disorder or disease are combined with the probe to evaluate any deviation from the standard profile, and a therapeutic agent is administered and effects are monitored to generate a

treatment profile. Efficacy is evaluated by determining whether the expression progresses toward or returns to the standard normal pattern. Treatment profiles may be generated over a period of several days or several months. Statistical methods well known to those skilled in the art may be used to determine the significance of such therapeutic agents.

[0725] The polynucleotides are also useful for identifying individuals from minute biological samples, for example, by matching the RFLP pattern of a sample's DNA to that of an individual's DNA. The polynucleotides of the present invention can also be used to determine the actual base-by-base DNA sequence of selected portions of an individual's genome. These sequences can be used to prepare PCR primers for amplifying and isolating such selected DNA, which can then be sequenced. Using this technique, an individual can be identified through a unique set of DNA sequences. Once a unique ID database is established for an individual, positive identification of that individual can be made from extremely small tissue samples.

[0726] In a particular aspect, oligonucleotide primers derived from the dithp of the invention may be used to detect single nucleotide polymorphisms (SNPs). SNPs are substitutions, insertions and deletions that are a frequent cause of inherited or acquired genetic disease in humans. Methods of SNP detection include, but are not limited to, single-stranded conformation polymorphism (SSCP) and fluorescent SSCP (fSSCP) methods. In SSCP, oligonucleotide primers derived from dithp are used to amplify DNA using the polymerase chain reaction (PCR). The DNA may be derived, for example, from diseased or normal tissue, biopsy samples, bodily fluids, and the like. SNPs in the DNA cause differences in the secondary and tertiary structures of PCR products in single-stranded form, and these differences are detectable using gel electrophoresis in non-denaturing gels. In fSSCP, the oligonucleotide primers are fluorescently labeled, which allows detection of the amplicons in high-throughput equipment such as DNA sequencing machines. Additionally, sequence database analysis methods, termed in silico SNP (is SNP), are capable of identifying polymorphisms by comparing the sequences of individual overlapping DNA fragments which assemble into a common consensus sequence. These computer-based methods filter out sequence variations due to laboratory preparation of DNA and sequencing errors using statistical models and automated analyses of DNA sequence chromatograms. In the alternative, SNPs may be detected and characterized by mass spectrometry using, for example, the high throughput MASSARRAY system (Sequenom, Inc., San Diego Calif.).

[0727] DNA-based identification techniques are critical in forensic technology. DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, semen, etc., can be amplified using, e.g., PCR, to identify individuals. (See, e.g., Erlich, H. (1992) *PCR Technology*, Freeman and Co., New York N.Y.). Similarly, polynucleotides of the present invention can be used as polymorphic markers.

[0728] There is also a need for reagents capable of identifying the source of a particular tissue. Appropriate reagents can comprise, for example, DNA probes or primers prepared from the sequences of the present invention that are specific for particular tissues. Panels of such reagents can identify

tissue by species and/or by organ type. In a similar fashion, these reagents can be used to screen tissue cultures for contamination.

[0729] The polynucleotides of the present invention can also be used as molecular weight markers on nucleic acid gels or Southern blots, as diagnostic probes for the presence of a specific mRNA in a particular cell type, in the creation of subtracted cDNA libraries which aid in the discovery of novel polynucleotides, in selection and synthesis of oligomers for attachment to an array or other support, and as an antigen to elicit an immune response.

#### [0730] Disease Model Systems Using dithp

[0731] The dithp of the invention or their mammalian homologs may be "knocked out" in an animal model system using homologous recombination in embryonic stem (ES) cells. Such techniques are well known in the art and are useful for the generation of animal models of human disease. (See, e.g., U.S. Pat. Nos. 5,175,383 and 5,767,337.) For example, mouse ES cells, such as the mouse 129/SvJ cell line, are derived from the early mouse embryo and grown in culture. The ES cells are transformed with a vector containing the gene of interest disrupted by a marker gene, e.g., the neomycin phosphotransferase gene (neo). Capocchi, M. R. (1989) *Science* 244:1288-1292. The vector integrates into the corresponding region of the host genome by homologous recombination. Alternatively, homologous recombination takes place using the Cre-loxP system to knockout a gene of interest in a tissue- or developmental stage-specific manner (Marth, J. D. (1996) *Clin Invest.* 97:1999-2002; Wagner, K. U. et al. (1997) *Nucleic Acids Res.* 25:4323-4330). Transformed ES cells are identified and microinjected into mouse cell blastocysts such as those from the C57BL/6 mouse strain. The blastocysts are surgically transferred to pseudopregnant dams, and the resulting chimeric progeny are genotyped and bred to produce heterozygous or homozygous strains. Transgenic animals thus generated may be tested with potential therapeutic or toxic agents.

[0732] The dithp of the invention may also be manipulated in vitro in ES cells derived from human blastocysts. Human ES cells have the potential to differentiate into at least eight separate cell lineages including endoderm, mesoderm, and ectodermal cell types. These cell lineages differentiate into, for example, neural cells, hematopoietic lineages, and cardiomyocytes (Thomson, J. A. et al. (1998) *Science* 282:1145-1147).

[0733] The dithp of the invention can also be used to create "knockin" humanized animals (pigs) or transgenic animals (mice or rats) to model human disease. With knockin technology, a region of dithp is injected into animal ES cells, and the injected sequence integrates into the animal cell genome. Transformed cells are injected into blastulae, and the blastulae are implanted as described above. Transgenic progeny or inbred lines are studied and treated with potential pharmaceutical agents to obtain information on treatment of a human disease. Alternatively, a mammal inbred to overexpress dithp, resulting, e.g., in the secretion of DITHP in its milk, may also serve as a convenient source of that protein (Janne, J. et al. (1998) *Biotechnol. Annu. Rev.* 45:55-74).

#### [0734] Screening Assays

[0735] DITHP encoded by polynucleotides of the present invention may be used to screen for molecules that bind to

or are bound by the encoded polypeptides. The binding of the polypeptide and the molecule may activate (agonist), increase, inhibit (antagonist), or decrease activity of the polypeptide or the bound molecule. Examples of such molecules include antibodies, oligonucleotides, proteins (e.g., receptors), or small molecules.

[0736] Preferably, the molecule is closely related to the natural ligand of the polypeptide, e.g., a ligand or fragment thereof, a natural substrate, or a structural or functional mimetic. (See, Coligan et al., (1991) *Current Protocols in Immunology* 1(2): Chapter 5.) Similarly, the molecule can be closely related to the natural receptor to which the polypeptide binds, or to at least a fragment of the receptor, e.g., the active site. In either case, the molecule can be rationally designed using known techniques.

[0737] Preferably, the screening for these molecules involves producing appropriate cells which express the polypeptide, either as a secreted protein or on the cell membrane. Preferred cells include cells from mammals, yeast, *Drosophila*, or *E. coli*. Cells expressing the polypeptide or cell membrane fractions which contain the expressed polypeptide are then contacted with a test compound and binding, stimulation, or inhibition of activity of either the polypeptide or the molecule is analyzed.

[0738] An assay may simply test binding of a candidate compound to the polypeptide, wherein binding is detected by a fluorophore, radioisotope, enzyme conjugate, or other detectable label. Alternatively, the assay may assess binding in the presence of a labeled competitor.

[0739] Additionally, the assay can be carried out using cell-free preparations, polypeptide/molecule affixed to a solid support, chemical libraries, or natural product mixtures. The assay may also simply comprise the steps of mixing a candidate compound with a solution containing a polypeptide, measuring polypeptide/molecule activity or binding, and comparing the polypeptide/molecule activity or binding to a standard.

[0740] Preferably, an ELISA assay using, e.g., a monoclonal or polyclonal antibody, can measure polypeptide level in a sample. The antibody can measure polypeptide level by either binding, directly or indirectly, to the polypeptide or by competing with the polypeptide for a substrate.

[0741] All of the above assays can be used in a diagnostic or prognostic context. The molecules discovered using these assays can be used to treat disease or to bring about a particular result in a patient (e.g., blood vessel growth) by activating or inhibiting the polypeptide/molecule. Moreover, the assays can discover agents which may inhibit or enhance the production of the polypeptide from suitably manipulated cells or tissues.

[0742] Transcript Imaging and Toxicological Testing

[0743] Another embodiment relates to the use of dithp to develop a transcript image of a tissue or cell type. A transcript image represents the global pattern of gene expression by a particular tissue or cell type. Global gene expression patterns are analyzed by quantifying the number of expressed genes and their relative abundance under given conditions and at a given time. (See, Sellhammer et al., "Comparative Gene Transcript Analysis," U.S. Pat. No. 5,840,484, expressly incorporated by reference herein.)

Thus a transcript image may be generated by hybridizing the polynucleotides of the present invention or their complements to the totality of transcripts or reverse transcripts of a particular tissue or cell type. In one embodiment, the hybridization takes place in high-throughput format, wherein the polynucleotides of the present invention or their complements comprise a subset of a plurality of elements on a microarray. The resultant transcript image would provide a profile of gene activity pertaining to human molecules for diagnostics and therapeutics.

[0744] Transcript images which profile dithp expression may be generated using transcripts isolated from tissues, cell lines, biopsies, or other biological samples. The transcript image may thus reflect dithp expression *in vivo*, as in the case of a tissue or biopsy sample, or *in vitro*, as in the case of a cell line.

[0745] Transcript images which profile dithp expression may also be used in conjunction with *in vitro* model systems and preclinical evaluation of pharmaceuticals, as well as toxicological testing of industrial and naturally-occurring environmental compounds. All compounds induce characteristic gene expression patterns, frequently termed molecular fingerprints or toxicant signatures, which are indicative of mechanisms of action and toxicity (Nwusyr, E. E. et al. (1999) *Mol. Carcinog.* 24:153-159; Steiner, S. and Anderson, N. L. (2000) *Toxicol. Lett.* 112-113:467-71, expressly incorporated by reference herein). If a test compound has a signature similar to that of a compound with known toxicity, it is likely to share those toxic properties. These fingerprints or signatures are most useful and refined when they contain expression information from a large number of genes and gene families. Ideally, a genome-wide measurement of expression provides the highest quality signature. Even genes whose expression is not altered by any tested compounds are important as well, as the levels of expression of these genes are used to normalized the rest of the expression data. The normalization procedure is useful for comparison of expression data after treatment with different compounds. While the assignment of gene function to elements of a toxicant signature aids in interpretation of toxicity mechanisms, knowledge of gene function is not necessary for the statistical matching of signatures which leads to prediction of toxicity. (See, for example, Press Release 00-02 from the National Institute of Environmental Health Sciences, released Feb. 29, 2000, available at <http://www.niehs.nih.gov/oc/news/toxchip.htm>). Therefore, it is important and desirable in toxicological screening using toxicant signatures to include all expressed gene sequences.

[0746] In one embodiment, the toxicity of a test compound is assessed by treating a biological sample containing nucleic acids with the test compound. Nucleic acids that are expressed in the treated biological sample are hybridized with one or more probes specific to the polynucleotides of the present invention, so that transcript levels corresponding to the polynucleotides of the present invention may be quantified. The transcript levels in the treated biological sample are compared with levels in an untreated biological sample. Differences in the transcript levels between the two samples are indicative of a toxic response caused by the test compound in the treated sample.

[0747] Another particular embodiment relates to the use of DITHP encoded by polynucleotides of the present invention

to analyze the proteome of a tissue or cell type. The term proteome refers to the global pattern of protein expression in a particular tissue or cell type. Each protein component of a proteome can be subjected individually to further analysis. Proteome expression patterns, or profiles, are analyzed by quantifying the number of expressed proteins and their relative abundance under given conditions and at a given time. A profile of a cell's proteome may thus be generated by separating and analyzing the polypeptides of a particular tissue or cell type. In one embodiment, the separation is achieved using two-dimensional gel electrophoresis, in which proteins from a sample are separated by isoelectric focusing in the first dimension, and then according to molecular weight by sodium dodecyl sulfate slab gel electrophoresis in the second dimension (Steiner and Anderson, supra). The proteins are visualized in the gel as discrete and uniquely positioned spots, typically by staining the gel with an agent such as Coomassie Blue or silver or fluorescent stains. The optical density of each protein spot is generally proportional to the level of the protein in the sample. The optical densities of equivalently positioned protein spots from different samples, for example, from biological samples either treated or untreated with a test compound or therapeutic agent, are compared to identify any changes in protein spot density related to the treatment. The proteins in the spots are partially sequenced using, for example, standard methods employing chemical or enzymatic cleavage followed by mass spectrometry. The identity of the protein in a spot may be determined by comparing its partial sequence, preferably of at least 5 contiguous amino acid residues, to the polypeptide sequences of the present invention. In some cases, further sequence data may be obtained for definitive protein identification.

[0748] A proteomic profile may also be generated using antibodies specific for DITHP to quantify the levels of DITHP expression. In one embodiment, the antibodies are used as elements on a microarray, and protein expression levels are quantified by exposing the microarray to the sample and detecting the levels of protein bound to each array element (Lueking, A. et al. (1999) *Anal. Biochem.* 270:103-11; Mendoza, L. G. et al. (1999) *Biotechniques* 27:778-88). Detection may be performed by a variety of methods known in the art, for example, by reacting the proteins in the sample with a thiol- or amino-reactive fluorescent compound and detecting the amount of fluorescence bound at each array element.

[0749] Toxicant signatures at the proteome level are also useful for toxicological screening, and should be analyzed in parallel with toxicant signatures at the transcript level. There is a poor correlation between transcript and protein abundances for some proteins in some tissues (Anderson, N. L. and Seilhamer, J. (1997) *Electrophoresis* 18:533-537), so proteomic toxicant signatures may be useful in the analysis of compounds which do not significantly affect the transcript image, but which alter the proteomic profile. In addition, the analysis of transcripts in body fluids is difficult, due to rapid degradation of mRNA, so proteomic profiling may be more reliable and informative in such cases.

[0750] In another embodiment, the toxicity of a test compound is assessed by treating a biological sample containing proteins with the test compound. Proteins that are expressed in the treated biological sample are separated so that the amount of each protein can be quantified. The amount of

each protein is compared to the amount of the corresponding protein in an untreated biological sample. A difference in the amount of protein between the two samples is indicative of a toxic response to the test compound in the treated sample. Individual proteins are identified by sequencing the amino acid residues of the individual proteins and comparing these partial sequences to the DITHP encoded by polynucleotides of the present invention.

[0751] In another embodiment, the toxicity of a test compound is assessed by treating a biological sample containing proteins with the test compound. Proteins from the biological sample are incubated with antibodies specific to the DITHP encoded by polynucleotides of the present invention. The amount of protein recognized by the antibodies is quantified. The amount of protein in the treated biological sample is compared with the amount in an untreated biological sample. A difference in the amount of protein between the two samples is indicative of a toxic response to the test compound in the treated sample.

[0752] Transcript images may be used to profile dithp expression in distinct tissue types. This process can be used to determine human molecule activity in a particular tissue type relative to this activity in a different tissue type. Transcript images may be used to generate a profile of dithp expression characteristic of diseased tissue. Transcript images of tissues before and after treatment may be used for diagnostic purposes, to monitor the progression of disease, and to monitor the efficacy of drug treatments for diseases which affect the activity of human molecules.

[0753] Transcript images of cell lines can be used to assess human molecule activity and/or to identify cell lines that lack or misregulate this activity. Such cell lines may then be treated with pharmaceutical agents, and a transcript image following treatment may indicate the efficacy of these agents in restoring desired levels of this activity. A similar approach may be used to assess the toxicity of pharmaceutical agents as reflected by undesirable changes in human molecule activity. Candidate pharmaceutical agents may be evaluated by comparing their associated transcript images with those of pharmaceutical agents of known effectiveness.

#### [0754] Antisense Molecules

[0755] The polynucleotides of the present invention are useful in antisense technology. Antisense technology or therapy relies on the modulation of expression of a target protein through the specific binding of an antisense sequence to a target sequence encoding the target protein or directing its expression. (See, e.g., Agrawal, S., ed. (1996) *Antisense Therapeutics*, Humana Press Inc., Totowa N.J.; Alama, A. et al. (1997) *Pharmacol. Res.* 36(3):171-178; Crooke, S. T. (1997) *Adv. Pharmacol.* 40:149; Sharma, H. W. and R. Narayanan (1995) *Bioessays* 17(12):1055-1063; and Lavrosky, Y. et al. (1997) *Biochem. Mol. Med.* 62(1):11-22.) An antisense sequence is a polynucleotide sequence capable of specifically hybridizing to at least a portion of the target sequence. Antisense sequences bind to cellular mRNA and/or genomic DNA, affecting translation and/or transcription. Antisense sequences can be DNA, RNA, or nucleic acid mimics and analogs. (See, e.g., Rossi, J. J. et al. (1991) *Antisense Res. Dev.* 1(3):285-288; Lee, R. et al. (1998) *Biochemistry* 37(3):906-1010; Partridge, W. M. et al. (1995) *Proc. Natl. Acad. Sci. USA* 92(12):5592-5596; and Nielsen, P. E. and Haasima, G. (1997) *Chem. Soc. Rev.*

96:73-78.) Typically, the binding which results in modulation of expression occurs through hybridization or binding of complementary base pairs. Antisense sequences can also bind to DNA duplexes through specific interactions in the major groove of the double helix.

[0756] The polynucleotides of the present invention and fragments thereof can be used as antisense sequences to modify the expression of the polypeptide encoded by dithp. The antisense sequences can be produced *ex vivo*, such as by using any of the ABI nucleic acid synthesizer series (Applied Biosystems) or other automated systems known in the art. Antisense sequences can also be produced biologically, such as by transforming an appropriate host cell with an expression vector containing the sequence of interest. (See, e.g., Agrawal, *supra*.)

[0757] In therapeutic use, any gene delivery system suitable for introduction of the antisense sequences into appropriate target cells can be used. Antisense sequences can be delivered intracellularly in the form of an expression plasmid which, upon transcription, produces a sequence complementary to at least a portion of the cellular sequence encoding the target protein. (See, e.g., Slater, J. E., et al. (1998) *J. Allergy Clin. Immunol.* 102(3):469-475; and Scanlon, K. J., et al. (1995) 9(13):1288-1296.) Antisense sequences can also be introduced intracellularly through the use of viral vectors, such as retrovirus and adeno-associated virus vectors. (See, e.g., Miller, A. D. (1990) *Blood* 76:271; Ausubel F. M. et al. (1995) *Current Protocols in Molecular Biology*, John Wiley & Sons, New York N.Y.; Uckert, W. and W. Walther (1994) *Pharmacol. Ther.* 63(3):323-347.) Other gene delivery mechanisms include liposome-derived systems, artificial viral envelopes, and other systems known in the art. (See, e.g., Rossi, J. J. (1995) *Br. Med. Bull.* 51(1):217-225; Boado, R. J. et al. (1998) *J. Pharm. Sci.* 87(11):1308-1315; and Morris, M. C. et al. (1997) *Nucleic Acids Res.* 25(14):2730-2736.)

[0758] Expression

[0759] In order to express a biologically active DITHP, the nucleotide sequences encoding DITHP or fragments thereof may be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for transcriptional and translational control of the inserted coding sequence in a suitable host. Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding DITHP and appropriate transcriptional and translational control elements. These methods include *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. (See, e.g., Sambrook, *supra*, Chapters 4, 8, 16, and 17; and Ausubel, *supra*, Chapters 9, 10, 13, and 16.)

[0760] A variety of expression vector/host systems may be utilized to contain and express sequences encoding DITHP. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with viral expression vectors (e.g., baculovirus); plant cell systems transformed with viral expression vectors (e.g., cauliflower mosaic virus, CaMV, or tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal (mammalian) cell systems. (See, e.g., Sambrook, *supra*; Ausubel, 1995, *supra*, Van

Hecke, G. and S. M. Schuster (1989) *J. Biol. Chem.* 264:5503-5509; Bitter, G. A. et al. (1987) *Methods Enzymol.* 153:516-544; Scorer, C. A. et al. (1994) *Bio/Technology* 12:181-184; Engelhardt, E. K. et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:3224-3227; Sandig, V. et al. (1996) *Hume Gene Ther.* 7:1937-1945; Takamatsu, N. (1987) *EMBO J.* 6:307-311; Coruzzi, G. et al. (1984) *EMBO J.* 3:1671-1680; Broglie, R. et al. (1984) *Science* 224:838-843; Winter, J. et al. (1991) *Results Probl. Cell Differ.* 17:85-105; *The McGraw Hill Yearbook of Science and Technology* (1992) McGraw Hill, New York N.Y., pp. 191-196; Logan, J. and T. Shenk (1984) *Proc. Natl. Acad. Sci. USA* 81:3655-3659; and Harrington, J. J. et al. (1997) *Natl. Genet.* 15:345-355.) Expression vectors derived from retroviruses, adenoviruses, or herpes or vaccinia viruses, or from various bacterial plasmids, may be used for delivery of nucleotide sequences to the targeted organ, tissue, or cell population. (See, e.g., Di Nicola, M. et al. (1998) *Cancer Gen. Ther.* 5(6):350-356; Yu, M. et al. (1993) *Proc. Natl. Acad. Sci. USA* 90(13):6340-6344; Buller, R. M. et al. (1985) *Nature* 317(6040):813-815; McGregor, D. P. et al. (1994) *Mol. Immunol.* 31(3):219-226; and Verma, I. M. and N. Somia (1997) *Nature* 389:239-242.) The invention is not limited by the host cell employed.

[0761] For long term production of recombinant proteins in mammalian systems, stable expression of DITHP in cell lines is preferred. For example, sequences encoding DITHP can be transformed into cell lines using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Any number of selection systems may be used to recover transformed cell lines. (See, e.g., Wigler, M. et al. (1977) *Cell* 11:223-232; Lowy, I. et al. (1980) *Cell* 22:817-823; Wigler, M. et al. (1980) *Proc. Natl. Acad. Sci. USA* 77:3567-3570; Colbere-Garapin, F. et al. (1981) *J. Mol. Biol.* 150:1-14; Hartman, S. C. and R. C. Mulligan (1988) *Proc. Natl. Acad. Sci. USA* 85:8047-8051; Rhodes, C. A. (1995) *Methods Mol. Biol.* 55:121-131.)

[0762] Therapeutic Uses of dithp

[0763] The dithp of the invention may be used for somatic or germline gene therapy. Gene therapy may be performed to (i) correct a genetic deficiency (e.g., in the cases of severe combined immunodeficiency (SCID)-X1 disease characterized by X-linked inheritance (Cavazzana-Calvo, M. et al. (2000) *Science* 288:669-672), severe combined immunodeficiency syndrome associated with an inherited adenosine deaminase (ADA) deficiency (Blaese, R. M. et al. (1995) *Science* 270:475-480; Bordignon, C. et al. (1995) *Science* 270:470-475), cystic fibrosis (Zabner, R. et al. (1993) *Cell* 75:207-216; Crystal, R. G. et al. (1995) *Hum. Gene Therapy* 6:643-666; Crystal, R. G. et al. (1995) *Hum. Gene Therapy* 6:667-703), thalassemias, familial hypercholesterolemia, and hemophilia resulting from Factor VIII or Factor IX deficiencies (Crystal, R. G. (1995) *Science* 270:404-410; Verma, I. M. and Somia, N. (1997) *Nature* 389:239-242)), (ii) express a conditionally lethal gene product (e.g., in the case of cancers which result from unregulated cell proliferation), or (iii) express a protein which affords protection against intracellular parasites (e.g., against human retroviruses, such as human immunodeficiency virus (HIV) (Baltimore, D. (1988) *Nature* 335:395-396; Poeschl, E. et al. (1996) *Proc. Natl. Acad. Sci. USA* 93:11395-11399), hepatitis B or C virus (HBV, HCV); fungal parasites, such as *Candida albicans* and *Paracoccidioides brasiliensis*; and